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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS :	Flugelman		
SERIAL NUMBER :	09/620,227	**EXAMINER :**	Thomas C. Barrett
FILING DATE :	July 20, 2000	**ART UNIT :**	3738
FOR :	ARTIFICIAL VASCULAR GRAFTS, AND METHODS OF PRODUCING AND USING SAME		

MAIL STOP RCE
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DECLARATION OF DR. MOSHE Y. FLUGELMAN UNDER 37 C.F.R. §1.132

I, Moshe Flugelman, declare and state that:

1. I am the sole inventor of the subject matter of the above-referenced patent application.

2. I am the Chief Executive Officer and founder of Multi-Gene Vascular Systems, Ltd. (the Assignee of this application), and am the Chief of In-patient Cardiology in the Department of Cardiovascular Medicine at the Lady Davis Carmel Medical Center in Haifa, Israel. A principal aspect of my research has been studying vascular biology in the context of gene therapy for treating a variety of cardiovascular disorders. Of particular interest is the use of multi-gene therapy in creating bio-engineered grafts with improved patency and lifetime for use in peripheral vascular disease. This work forms part of the subject matter of the present invention.

3. I have reviewed the final Office Action dated December 13, 2005. I understand that claims 1-12, 19-25, and 51-55 are pending. I further understand that claims 11, 19-25, and 51 are rejected under 35 U.S.C. § 103(a) as obvious in view of U.S. Patent No. 5,785,965 ("Pratt") in combination with Nakamura et al. (1999) J. Biol. Chem., 274(32): 22476-83 ("Nakamura").

4. I have reviewed the accompanying amendment and the above-referenced application in conjunction with the cited references. I make this declaration to rebut the Examiner's assertions, with which I do not agree. The Examiner asserts that, as of the date of invention it would have been obvious to one of ordinary skill in the art to make a vascular graft seeded with endothelial cells genetically transformed to express or over-express VEGF and UP50. *See* Office Action at pp. 3-4. For the reasons discussed below, it is my opinion that the claims as presented are not obvious in view of the cited references.

5. Claim 11 depends directly from claim 1 and recites, in relevant part, an artificial vascular graft coated with a plurality of endothelial cells that have been genetically transformed with at least one sequence to express or over-express at least one endothelial cell proliferating growth factor and at least one sequence to express or over-express at least one cellular adherence factor, wherein the adherence factor is UP50 (also known as DANCE or Fibulin-5). While claims 19-25 are directed to methods of producing the artificial vascular graft seeded with endothelial cells genetically transformed to express or over-express a growth factor and a cellular adhesion factor, and claim 51 is directed to methods of producing the genetically transformed endothelial cells, claims 19-25 and 51 do not specifically recite genetically transformed endothelial cells that express or over-express UP50.

6. As described in detail in the instant specification, a primary objective of the present invention is to increase the patency and life-span of vascular grafts, which historically have failed within 7 years or less due to neointimal proliferation and/or thrombosis. *See* specification at ¶¶ 0002-0004. In order to accomplish this objective, the proliferative capacity of endothelial cells must be maintained in the lumen of the graft because endothelial cell coverage is critical for preventing thrombosis, preventing tissue in-growth, and increasing durability of these vascular grafts. *See* specification, ¶¶ 0061 and 0097. Thus, endothelial cell proliferation is a desirable event.

7. There is no teaching or suggestion in the references themselves that would have motivated one of ordinary skill in the art at the time of invention to combine the teachings of Pratt with the teachings of Nakamura to achieve the claimed invention. Pratt's objective is to provide an improved vascular graft seeded with genetically modified endothelial cells, which

facilitate rapid and complete endothelialization of the graft for increased patency and decreased intimal thickening. *See* Pratt at col. 1, lines 46-63; col. 2, lines 45-53; col. 4, lines 60-66; and col. 13, lines 31-41. Pratt teaches PTFE vascular grafts, wherein the lumen is seeded with endothelial cells transformed to express VEGF. *See* Pratt, Abstract; col. 2, lines 45-53; and col. 3, lines 29-42. Pratt also presents the results of implantation of a vascular graft in rabbits. Upon sacrifice of the rabbits at 8 hours, 14 days, and 28 days post-implant, Pratt observed that there was "no unusual adhesion, hematoma or seroma adhesion around the grafts." *See* Pratt, col. 12, lines 51-54. More importantly, Pratt indicates that the "grafts from all three groups were patent." *See id.* at lines 55-56. Thus, Pratt did not observe any problems with adhesion of the endothelial cells transformed with VEGF and seeded on the grafts. Accordingly, the reference is individually complete and functional in and of itself and there is no reason or motivation to substitute parts from another reference.

8. Pratt is fully aware of the problems with incomplete endothelialization and denudation as a result of sheer forces *in vivo*. For example, Pratt indicates that "[p]revious attempts have seeded endothelial cells onto synthetic grafts, but this results in incomplete endothelialization as well as denudation upon exposure to flow." *See* Pratt, col. 14, lines 5-7; *see also* col. 1, lines 49-53. Significantly, Pratt states that VEGF gene overexpression promotes endothelialization of the prosthetic vascular graft surface and graft healing, and that it is evident from such results that the invention provides for an improvement in the endothelialization of synthetic vascular grafts by the genetic modification of endothelial cells with VEGF. *See* Pratt, col. 14, lines 18-26. Thus, Pratt clearly indicates that the problems associated with incomplete endothelialization and subsequent denudation as a result of poor adhesion of cells due to sheer forces *in vivo* were solved by its invention.

9. In fact, contrary to the Examiner's assertion that it would be obvious to the skilled artisan to make a vascular graft of PTFE with a lumen seeded with endothelial cells (as taught by Pratt), and that the endothelial cells could be transformed to express UP50 (as taught by Nakamura), the references actually teach away from each other, and, thus, teach away from the suggested combination.

10. As previously discussed, *supra* at paragraph 7, Pratt teaches PTFE vascular grafts, wherein the lumen is seeded with endothelial cells transformed to express VEGF. *See* Pratt, Abstract; col. 2, lines 45-53; and col. 3, lines 29-42. Pratt's objective is to provide an improved vascular graft seeded with genetically modified endothelial cells, which facilitate rapid and complete endothelialization of the graft for increased patency and decreased intimal thickening. *See* Pratt at col. 1, lines 46-63; col. 2, lines 45-53; col. 4, lines 60-66; and col. 13, lines 31-41. Nakamura is an initial identification and characterization of DANCE (also known as UP50 and fibulin-5) expression in various cell types. When considered in its totality, Nakamura indicates that DANCE affects cell growth as a "brake," because it is observed that DANCE is underexpressed at the leading edge of regenerating endothelium in balloon-injured vessels, while increased expression is seen in the area of cell quiescence. *See* Nakamura at p. 22483, col. 1, fourth paragraph. Thus, and significantly, Nakamura teaches that overexpression of DANCE prevents vigorous proliferation of endothelial cells in the lumen. Accordingly, one of ordinary skill in the art at the time of invention would not have been motivated to combine Nakamura with Pratt to achieve the claimed invention, because the skilled artisan would have believed that such a combination would have rendered Pratt unsatisfactory for its intended purpose; namely, to provide an improved vascular graft seeded with genetically modified endothelial cells, which facilitate rapid and complete endothelialization of the graft.

11. As previously discussed, one of the objectives of the grafts as presently claimed is to increase graft patency and lifetime by preventing neointimal proliferation and thrombosis. One of ordinary skill in the art at the time of invention of the present claims would not have had a reasonable expectation of success in achieving this objective by seeding endothelial cells transformed with, or seeded in the presence of, a cell growth factor and an inhibitor of endothelial cell proliferation. However, this is exactly what has been accomplished by the instant application. Proceeding contrary to accepted wisdom at the time of invention, the instant application teaches that artificial grafts seeded with endothelial cells genetically altered to express or over-express a cell growth factor (such as VEGF) and a cellular adherence factor such as UP50 promotes the proliferation of endothelial cells, thereby increasing graft patency and lifetime.

12. In experiments performed in my laboratory, I have independently confirmed that UP50 partially inhibits both smooth muscle cells and endothelial cell proliferation (as suggested by Nakamura). Surprisingly, however, when a growth factor such as VEGF is added to the culture, endothelial cells rapidly proliferate while smooth muscle cells remain partially inhibited. Thus, in the presence of UP50, VEGF has a synergistic effect on endothelial cell proliferation, which effect is superior to endothelial cells transformed with VEGF alone. Data from my lab (attached hereto as Exhibit A) confirms that endothelial cells transfected to express VEGF alone (as done in Pratt) show increased detachment from the inner surface of the vascular graft upon exposure to flow, thereby increasing the likelihood of neointimal formation and eventual failure of the graft.

13. For example, the effect of UP50 on endothelial cell proliferation and reversal of the effect with the addition of VEGF can be seen in the figure attached hereto as Exhibit B. Primary endothelial cells were isolated from human saphenous vein. Cells were retrovirally transduced using a LXSN based viral vector encoding UP50. Additional study groups included – naive EC, $VEGF_{165}$ (a specific mitogen of endothelial cells) expressing EC, GFP expressing EC, UP50 and $VEGF_{165}$ expressing EC, UP50 expressing EC supplemented with exogenous $VEGF_{165}$. For proliferation assays we seeded 20,000 cell/well in a 24 wells plate pre-coated with gelatin. Cells were counted using a coulter counter at day 0, day 2, day 4, and day 5. The results show that UP50 inhibits endothelial cell proliferation, but that the supplementation of VEGF increases endothelial cell proliferation, such that the combination of UP50 and VEGF on endothelial cell proliferation is better than with VEGF alone.

14. Significantly, the effect of UP50 on smooth muscle cell proliferation, with no reversal of the effect upon the addition of bFGF, can be seen in the figure attached hereto as Exhibit C. In this study, primary smooth muscle cells were isolated from human saphenous vein. Cells were retrovirally transduced using a LXSN based viral vector encoding UP50. For control we used cells transduced with GFP. For the assay, we seeded 20,000 cell/well in a 24 well plate pre-coated with gelatin. Cells were counted using a coulter counter at baseline, day 2, day 6, and day 9. The cells were grown with bFGF (a strong mitogen of smooth muscle cells). The results unequivocally show that UP50 inhibits smooth muscle cell proliferation even in the presence of a strong mitogenic growth factor, such as bFGF. This finding is

significant because bFGF is abundantly present in places of vascular injury, and is therefore a key player in neointimal formation and thrombosis.

15. Smooth muscle cell proliferation is an unwanted biological response to vascular injury. It causes restenosis (re-occlusion) after stent deployment or balloon angioplasty, and also occurs at the site of suturing a vascular graft to the native artery (anastamotic site). Local over expression of UP50 at the site of anastamosis will inhibit smooth muscle cell proliferation and reduce the unwanted biological phenomenon of restenosis at the site of anastamosis. Thus, local expression of UP50 alone can improve performance of grafts seeded with endothelial cells genetically transformed to express or over-express UP50. However, since we want to enhance coverage by endothelial cells, the addition of VEGF to the cells will achieve the goal of increasing and maintaining proliferative capacity of the endothelial cells. The inhibition of smooth muscle cell proliferation is thus achieved by two mechanisms: UP50 inhibits smooth muscle cell proliferation directly, while the combination of VEGF and UP50 selectively permits rapid endothelialization for complete coverage, thereby reducing thrombosis and neointimal proliferation. Such results are superior and unexpected over those grafts taught by the prior art.

16. Accordingly, in view of the foregoing discussion and data, it is my opinion that the combination of Pratt and Nakamura is improper, and that claims 11, 19-25, and 51 are unobvious in view of the combination. Therefore, it is requested that the Examiner withdraw the rejections under 35 U.S.C. § 103.

17. I further declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. § 1001 and that willful false statements may jeopardize the validity of this application and any patent issuing therefrom.

Respectfully submitted,

Moshe Y. Flugelman, M.D.

Signed at Haifa, Israel this 2nd day of April, 2006

Enclosures: Exhibits A-C